

# NORADRENALINE: FATE AND CONTROL OF ITS BIOSYNTHESIS

by

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When I joined the National Institute of Mental Health in 1955, I began to think of an appropriate problem on which to work. In reading the literature I was surprised to learn that very little was known about the metabolism of noradrenaline and adrenaline. In 1946 von Euler (1) isolated and identified noradrenaline in the sympathetic nervous system and was later to develop sensitive methods for measuring this catecholamine in tissues (2). In 1954 I had been working on the *in vivo* (3) and *in vitro* (4) metabolism of amphetamines and compounds related in structure to catecholamines. Because of this background, I decided to work on the metabolism of noradrenaline and adrenaline.

Just as this work was begun, Armstrong et al. (5) identified 3-methoxy-4-hydroxymandelic acid in the urine of subjects with adrenaline-forming tumors. This observation immediately suggested that catecholamines might undergo an O-methylation reaction. Cantoni had shown that S-adenosylmethionine

Table 1. Enzymatic O-Methylation of Catecholamines

Substrate	O-Methoxy Product Formed in $m\mu$ moles
1-Adrenaline	59
1-Adrenaline (AMe omitted)	0
1-Adrenaline ( $MgCl_2$ omitted)	4
dl-N-Methyl-4-hydroxy phenylethanolamine	0
d-Adrenaline	62
1-Noradrenaline	63
dl-Octopamine	0
Dopamine	60
Tyramine	0
Dopa	63

Catecholamines or other amines (0.3  $\mu$ mole) were incubated at 37° C with partially purified catechol-O-methyltransferase from rat liver, 50  $\mu$ moles pH 7.8 phosphate buffer, 150  $\mu$ moles S-adenosylmethionine (AMe) 10  $\mu$ moles magnesium chloride in a final volume of 1 ml for 30 minutes. When adrenaline, noradrenaline or dopamine were used as substrates the O-methoxy derivatives were measured. With other substrates their disappearance was measured (9).

formed enzymatically from ATP and methionine can donate its methyl group to the nitrogen of nicotinamide (6) and it appeared possible that S-adenosylmethionine could donate its methyl group to one of the hydroxy groups of catecholamines. In the initial experiment, a rat liver fraction was incubated with ATP, methionine and  $Mg^{++}$  and adrenaline, and the disappearance of the catecholamine was measured (7). When these cofactors were added there was a marked disappearance of the catecholamine. When either cofactor was omitted no metabolism took place. The requirement for both ATP and methionine suggested that the liver extract was making S-adenosylmethionine. With S-adenosylmethionine instead of ATP and methionine, even greater metabolism of adrenaline occurred (Table 1). The O-methylated metabolite was isolated by solvent extraction and identified as 3-methoxy-4-hydroxyphenyl-2-methylamino ethanol (metanephrine). Metanephrine and normetanephrine were synthesized within two days after isolation by Senoh and Witkop at the NIH.

Rat urine and tissues were then examined by solvent extraction and paper chromatography for the normal occurrence of normetanephrine, metanephrine and 3-methoxy tyramine. All these compounds were present in brain, spleen

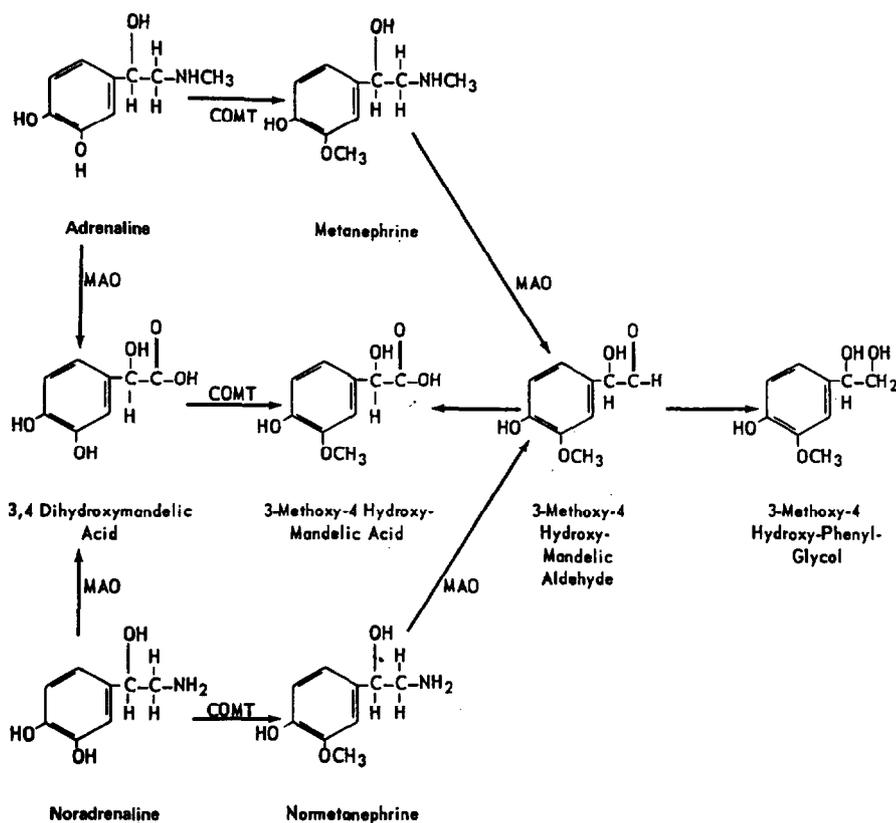


Fig. 1. Metabolism of noradrenaline and adrenaline. COMT is catechol-O-methyltransferase; MAO is monoamine oxidase.

and adrenal gland (8). Later, another O-methylated metabolite, 3-methoxy-4-hydroxyphenylglycol, was identified. The administration of noradrenaline, adrenaline or dopamine resulted in an elevated excretion of O-methylated amines, acid and alcohol metabolites. As a result of these experiments the scheme shown in Fig. 1 was proposed for the metabolism of noradrenaline and adrenaline. Dopamine undergoes an analogous pathway.

#### CATECHOL-O-METHYLTRANSFERASE (COMT)

The enzyme that O-methylates catecholamines was partially purified from rat liver and its properties studied (9). It requires  $Mg^{++}$  (Table 1), but other divalent ions such as  $Mn^{++}$ ,  $Co^{++}$ ,  $Zn^{++}$ ,  $Fe^{++}$ ,  $Cd^{++}$  and  $Ni^{++}$  could be substituted. S-adenosylmethionine is necessary as the methyl donor. All catechols examined were O-methylated by the enzyme, including adrenaline, noradrenaline, dopamine, dopa (Table 1), 3,4-dihydroxy mandelic acid, 3,4-dihydroxy phenylacetic acid, 3-hydroxy estradiol and ascorbic acid. Foreign catechols such as 3,4-dihydroxy ephedrine, 3,4-dihydroxy amphetamine and many substituted catechols and polyphenols can serve as substrates for COMT. Monophenols are not O-methylated (Table 1). O-methylation occurs mainly on the meta position. However, O-methylation *in vitro* occurs on both meta and para positions depending on the pH of the reaction mixture and the nature of the aromatic substrate (10).

The purified enzyme has a molecular weight of approximately 24,000 (11). At least two separate forms of the enzyme have been identified on starch block electrophoresis (12). The enzyme can be inhibited by polyphenols (13), 3-hydroxy estradiol (14) and tropolone (15). The administration *in vivo* of COMT inhibitors results in a small, but definite, prolongation of the physiological effects of noradrenaline (16).

COMT is present in all mammalian species examined (9) and exists also in some plants (17). Of all animal tissues, the liver and kidney exhibit highest activity. Unequally distributed in different regions of the brain, the enzyme's highest activity is present in the area postrema, and lowest activity is in the cerebellar cortex (18). Catechol-O-methyltransferase occurs mainly in the soluble fraction of the cell, but small amounts are present in fat cell membranes (19) and in microsomes (20). COMT acts on catecholamines mainly outside the neurone, whereas monoamine oxidase, the other major enzyme for catecholamine metabolism, is localized mainly within the neurone. However, small amounts of COMT are present in the sympathetic nerves of the nictitating membrane and the vas deferens (22). COMT is involved mainly in the metabolism of catecholamines released into the circulation (23) and in the inactivation of noradrenaline in tissues with sparse adrenergic innervation (24). It also appears to be associated with an extraneural uptake mechanism (25). Recently we have observed that COMT is present within mammalian erythrocytes. This provided an easily available tissue to examine this enzyme in man. The activity of COMT in erythrocytes is reduced in women with primary affective disorders (27).

The discovery of COMT led to the description of other methyltransferases involved in biogenic amine metabolism: histamine-N-methyltransferase (28), hydroxyindole-O-methyltransferase (29), phenylethanolamine-N-methyltransferase (30), and a nonspecific methyltransferase (31).

#### UPTAKE OF NORADRENALINE BY SYMPATHETIC NERVES

Soon after the work on O-methylation was begun, the distribution of H<sup>3</sup>-adrenaline in animal tissues was investigated. Fortunately, Seymour Kety arranged for the synthesis of tritiated noradrenaline and adrenaline of high specific activity labeled on a 7 position. This made possible the administration of physiological amounts of the neurotransmitter and a study of the localization and metabolism of the circulating catecholamine. In collaboration with Weil-Malherbe and Whitby, specific methods for the measurement of adrenaline, noradrenaline and its O-methylated metabolites in tissues were developed. After the intravenous injection of H<sup>3</sup>-adrenaline (32) or H<sup>3</sup>-noradrenaline (33) in cats, these catecholamines were rapidly and unequally distributed in tissues. The amines were selectively taken up in tissues heavily innervated with sympathetic nerves (heart, spleen). Since negligible amounts of H<sup>3</sup>-catecholamines were present in the brain, a blood brain barrier to these compounds was indicated. O-methylated metabolites, H<sup>3</sup>-metanephrine and H<sup>3</sup>-normetanephrine, also occurred in tissues. When tissues were examined two hours following the administration of the catecholamines, long after the physiological effects had disappeared, they were found to have almost the same levels of H<sup>3</sup>-adrenaline and H<sup>3</sup>-noradrenaline as those found after two minutes. These experiments suggested that noradrenaline and adrenaline were taken up and retained in tissues in a physiologically inactive form. The selective binding of the catecholamines by tissues with a high adrenergic innervation pointed to the sympathetic nerves as the sites of retention. To examine this possibility the superior cervical ganglia of cats were removed unilaterally and sufficient time (7 days) was allowed to elapse for complete degeneration of the sympathetic

Table 2. Lack of Uptake of H<sup>3</sup>-Noradrenaline after Chronic Denervation of the Sympathetic Nerves

	<i>Chronic Denervation</i>		<i>Acute Denervation</i>	
	Denervated	Innervated	Denervated	Innervated
Salivary Gland	5	42	76	89
Lachrymal Gland	3	45	—	—
Retractor Muscle	2	11	13	13
Ocular Muscle	6	48	25	26

Right superior cervical ganglia were removed from 6 cats. After 7 days cats were given 25  $\mu\text{g}/\text{kg}$  H<sup>3</sup>-noradrenaline and the H<sup>3</sup>-catecholamine assayed in innervated and denervated structures one hour later. In the acute denervation experiments right superior ganglia were removed 15 minutes before the administration of H<sup>3</sup>-noradrenaline. Results are expressed as  $\mu\text{g}$  H<sup>3</sup>-noradrenaline per gm tissue (34).

nerve fibers.  $H^3$ -noradrenaline was then given intravenously and the animals were killed one hour later and the  $H^3$ -catecholamine content was examined in structures innervated by the sympathetic cervical ganglia (34). There was a sharp reduction in the uptake of  $H^3$ -noradrenaline in the chronically denervated structures (Table 2). These results made it apparent that sympathetic nerve endings take up and retain the circulating catecholamine.

To localize the intraneuronal site of the noradrenaline retention, combined electron microscopy and autoradiography were carried out.  $H^3$ -noradrenaline was injected; 30 minutes later the pineal was prepared for autoradiography and electron microscopy (35). The pineal gland was chosen because of its rich sympathetic innervation. Electron microscopy showed a striking localization of photographic grains overlying non-myelinated axons which contained granulated vesicles of about 500 Å.

With Potter attempts were made to isolate the dense core vesicles associated with the  $H^3$ -noradrenaline (36, 37). Previously von Euler and Hillarp had isolated a high speed noradrenaline-containing granular fraction from bovine splenic nerves (38). Again,  $H^3$ -noradrenaline was injected in rats and subcellular fractions of the heart and other tissues were separated in a continuous sucrose gradient (36). The predominant peak of the  $H^3$ -noradrenaline together with the endogenous catecholamine coincided with the

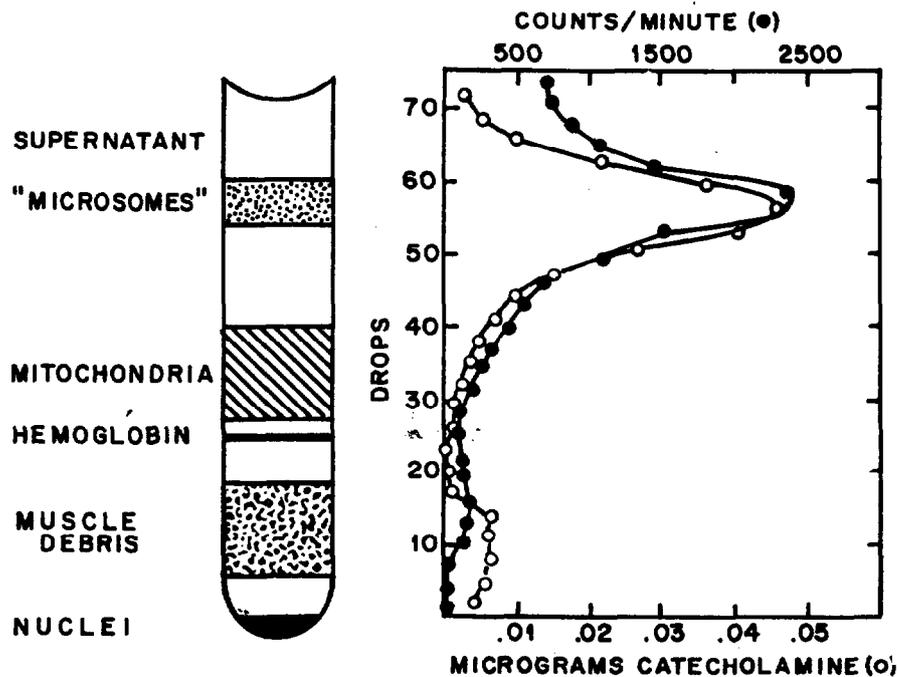


Fig. 2. Subcellular distribution of noradrenaline in the rat heart. Sprague-Dawley male rats were given 50  $\mu c$   $H^3$ -noradrenaline and were killed 30 minutes later. The hearts were rapidly removed and homogenized in isotonic sucrose. A portion was layered on an exponential sucrose gradient and centrifuged in a Spinco preparative centrifuge using an SW39 rotor for 30 minutes (36). Drops were collected with a needle through the bottom of the tube and assayed for  $H^3$  and endogenous noradrenaline.

“microsomal band” (Fig. 2). The noradrenaline containing particles had no pressor action unless they were lysed in dilute acid, suggesting that the catecholamine was bound. In addition to  $H^3$ -noradrenaline, the microsomal peak also contained large amounts of dopamine- $\beta$ -oxidase (37), the enzyme that converts dopamine to noradrenaline. Further attempts to purify noradrenaline containing vesicles were unsuccessful.

The ability to take up and store  $H^3$ -noradrenaline enabled Hertting and me to label the neurotransmitter in the nerve endings of tissues and to study its fate on liberation from sympathetic nerves (39). Cats were given  $H^3$ -noradrenaline; the spleen, containing nerve endings labelled with  $H^3$ -noradrenaline, was perfused; the splenic nerve was stimulated, as described by Brown and Gillespie (40); and the radioactive catecholamine and its metabolites were measured in the venous outflow. After each series of stimulations a marked increase occurred in the concentration of  $H^3$ -noradrenaline in the venous outflow. There was also a small but measurable elevation of the O-methylated metabolite, normetanephrine, but no increase in deaminated metabolites. From these experiments we concluded that noradrenaline liberated from the nerve terminals was inactivated by several mechanisms. Part is discharged into the bloodstream; part is O-methylated by COMT, and part is taken up by the nerve terminals. Reuptake of noradrenaline by sympathetic neurones was examined in experiments performed with Rosell and Kopin using the vascular bed of the dog gracilis muscle in situ (41). The sympathetic nerves of the gracilis muscle were labeled by an infusion of  $H^3$ -noradrenaline, and the discharge of  $H^3$ -noradrenaline measured after nerve stimulation. When the vasomotor nerves were stimulated, an initial reduction in the outflow of  $H^3$ -noradrenaline was followed by a rise in outflow of the radioactive catecholamine (Fig. 3). The lag in the outflow was due to an increase in vascular

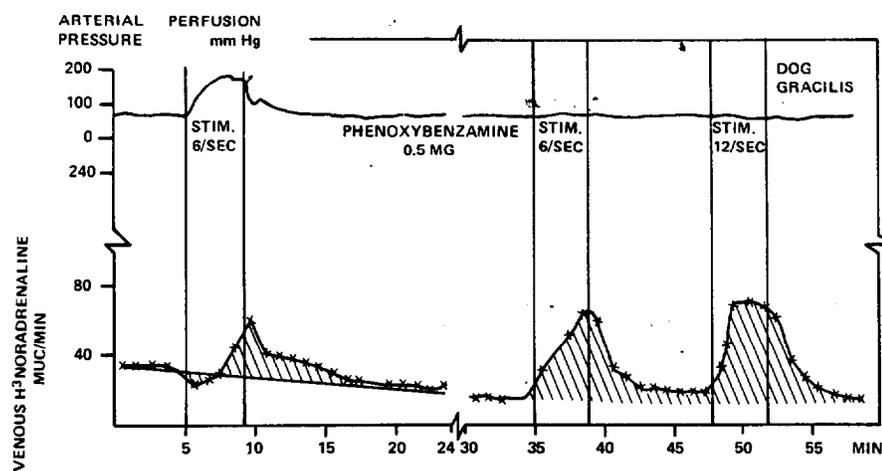


Fig. 3.

Uptake and release of  $H^3$ -noradrenaline in dog gracilis muscle. Dog gracilis muscle was perfused with  $H^3$ -noradrenaline as described by Rosell, Kopin and Axelrod (41). Peripheral resistance and venous outflow of  $H^3$ -noradrenaline was measured during sympathetic nerve stimulation, before and after treatment with phenoxybenzamine.

resistance. This observation indicates a reduced capacity of the vascular bed to carry away the released noradrenaline. After the stimulus was ended, decline in  $H^3$ -noradrenaline outflow and return of the peripheral resistance were parallel. To block the constriction of the vascular bed, dogs were pretreated with phenoxybenzamine, an adrenergic blocking agent shown to inhibit reuptake of noradrenaline. Vasomotor stimulation resulted in an immediate and larger increase in noradrenaline outflow. The larger and immediate outflow of noradrenaline was due to a blockade of noradrenaline reuptake by phenoxybenzamine. It was concluded from this and other experiments that reuptake by sympathetic nerves was a major mechanism for terminating the actions of the neurotransmitter noradrenaline. Subsequent work by several investigators, particularly Iversen (42, 43) described the properties of the neuronal uptake mechanism. It obeys saturation kinetics of the Michaelis-Menton type: it is stereospecific for the l-isomer of noradrenaline and requires  $Na^+$ . Many other amines structurally related to noradrenaline can be taken up and stored in sympathetic nerves by a neuronal uptake process. The fate of noradrenaline at the sympathetic nerve terminal and circulation is shown in Fig. 4.

Noradrenaline can also be taken up by an extraneuronal process (44, 45) which has been shown to be similar to Iversen's Uptake 2 (46). This uptake

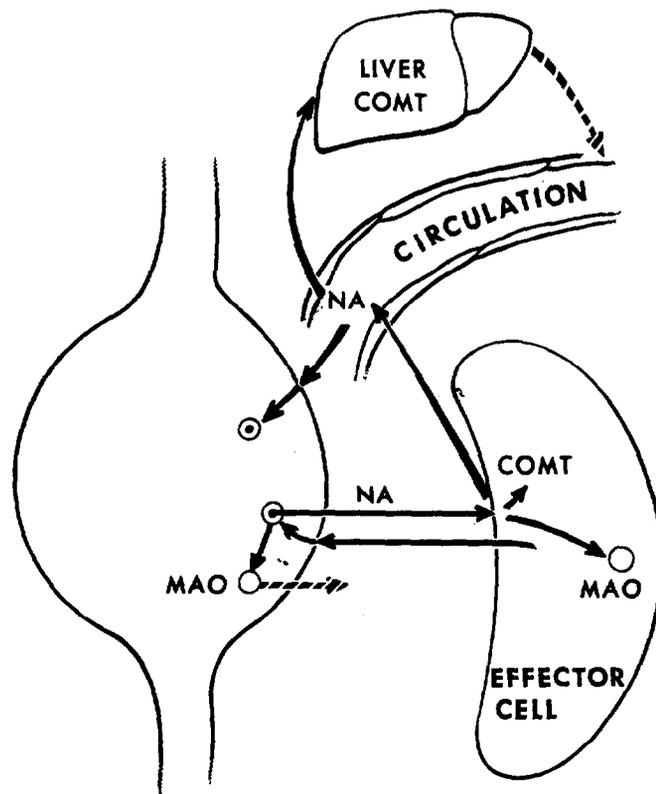


Fig. 4. Fate of noradrenaline (NA) at a varicosity of the sympathetic nerve terminal. COMT is catechol-O-methyltransferase; MAO is monoamine oxidase.

is inhibited by adrenergic blocking agents and normetanephrine (25). Compounds such as isoproterenol which have a low affinity for intraneural uptake and a high affinity for extraneural uptake may be inactivated by the later process. Extraneural uptake operates at all concentrations of catecholamines (47) and serves to transport amines into non-neuronal tissues in which they are subsequently metabolized.

#### EFFECT OF DRUGS ON NEURONAL UPTAKE

The ability of the sympathetic nerves to take up  $H^3$ -catecholamines provided a relatively simple technique for studying the effect of a variety of drugs acting on the sympathetic nervous system. In early experiments of this kind, mice were treated with a variety of drugs and the rate of disappearance of  $H^3$ -adrenaline was measured (48). A wide variety of drugs (imipramine, chlorpromazine, cocaine, reserpine, amphetamine, tyramine) increased the rate of disappearance of the catecholamine. Such experiments suggested that these drugs might increase the rate of metabolism by interfering with the binding and/or uptake of the catecholamines, thus exposing them to enzymatic attack by COMT or monoamine oxidase. This suggestion was supported by the observation that the catechol quercitrin markedly slowed catecholamine metabolism *in vivo*, presumably by inhibiting COMT.

The experiments that followed were more direct. Cats were treated with cocaine, and then  $H^3$ -noradrenaline was injected intravenously. One hour later, heart, spleen and adrenal gland were examined for  $H^3$ -noradrenaline (49). Cats pretreated with cocaine showed a dramatic decrease in tissue  $H^3$ -noradrenaline. In addition, there was a sharp elevation in plasma levels of  $H^3$ -noradrenaline in cocaine-treated animals. This experiment revealed that cocaine markedly reduces the uptake of noradrenaline in tissues, presumably the sympathetic neurone. The inhibition of uptake by cocaine thus raised the extraneural concentration of noradrenaline (as reflected by the elevated level in plasma catecholamine). By blocking uptake into the nerves, cocaine caused an elevated concentration of noradrenaline to reach the receptor (Fig. 5), and this explains the effect of the drug and denervation of sympathetic nerves in producing supersensitivity.

Experiments similar to those described with cocaine were carried out with

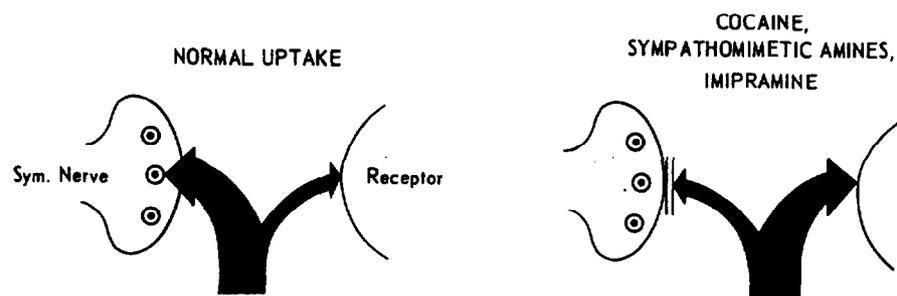


Fig. 5.  
Effect of drugs on uptake of noradrenaline at the sympathetic nerve terminal.

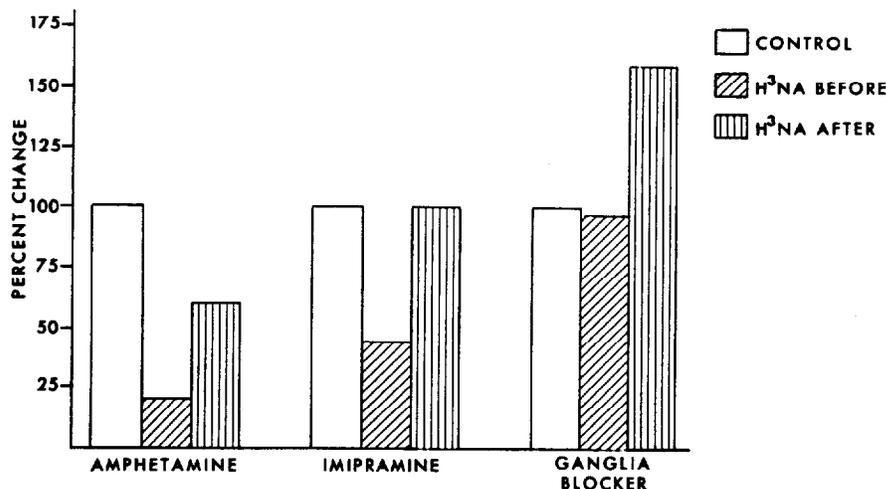


Fig. 6. Effect of drugs in uptake and release of H<sup>3</sup>-noradrenaline in the rat heart. Rats were given 15  $\mu$ c H<sup>3</sup>-noradrenaline 30 minutes before or after the administration of drugs and killed 24 hours later. The hearts were examined for H<sup>3</sup>-noradrenaline remaining (52, 54). The ganglia blocker was chlorisondamine.

other drugs (50, 51). The following compounds lowered the concentration of H<sup>3</sup>-noradrenaline in tissues: imipramine, chlorpromazine, tyramine, amphetamine, guanethedine, reserpine and phenoxybenzamine. All of these drugs also elevated the initial blood level of the H<sup>3</sup> catecholamine. Such observations indicate that these drugs also interfere with the uptake of noradrenaline into the adrenergic neurone.

In addition to blocking uptake, these drugs could also prevent the storage or release of the bound H<sup>3</sup>-noradrenaline. If a drug prevents noradrenaline uptake, it should lower the tissue levels of H<sup>3</sup>-noradrenaline only when given before the H<sup>3</sup> catecholamine. If it reduces the concentration when given after H<sup>3</sup>-noradrenaline, when the neurotransmitter is bound to tissue, then it releases the catecholamine. To distinguish between these two possibilities rats were given drugs before or after the intravenous injection of H<sup>3</sup>-noradrenaline, and the amount of the H<sup>3</sup>-catecholamine in the heart was measured (52). Reserpine, amphetamine, and tyramine reduced the H<sup>3</sup>-catecholamine after it was bound. Pretreatment with imipramine (Fig. 6) or chlorpromazine lowered the concentration of cardiac H<sup>3</sup>-noradrenaline only when given before H<sup>3</sup>-noradrenaline, indicating that these drugs blocked uptake but did not release the amine. Amphetamine caused a greater reduction when given before H<sup>3</sup>-noradrenaline than after (Fig. 6), indicating that it not only blocks uptake but also releases the catecholamine. Many of these observations were confirmed and extended through direct visualization of the sympathetic neurone by histofluorescent techniques (53).

H<sup>3</sup>-noradrenaline was also used to measure the effect of drugs in blocking the spontaneous release of the neurotransmitter. Long-lasting ganglionic blocking agents (chlorisondamine; Fig. 6) and bretylium inhibited the spontaneous

release of  $H^3$ -noradrenaline from the rat heart (54, 55). Decentralization of the superior cervical ganglion also slowed spontaneous release of  $H^3$ -noradrenaline, again demonstrating that nerve impulses cause a release of the  $H^3$ -noradrenaline (54).

#### UPTAKE, STORAGE, RELEASE AND METABOLISM OF $H^3$ -NORADRENALINE IN THE RAT BRAIN

In 1954 Vogt demonstrated the presence of noradrenaline in the brain and showed that it was unequally distributed (56). Drugs such as amphetamine and reserpine (56, 57, 58) lowered the tissue concentration of endogenous noradrenaline, whereas monoaminoxidase inhibitors elevated the level of the catecholamine (58). In our earlier work we were unable to study the disposition of noradrenaline in the brain because of its inability to cross the blood-brain barrier (33). In 1964 Jacques Glowinski devised a technique for introducing  $H^3$ -noradrenaline into the rat brain via the lateral ventricle (59). This provided a means of labeling the brain stores of noradrenaline and enabled us to study the fate of this compound in the brain and examine the effect of drugs. The initial concern was whether this exogenously administered  $H^3$ -noradrenaline mixed with the endogenous pool of brain catecholamines. We first examined the distribution of the  $H^3$ -noradrenaline in various brain areas. After an intraventricular injection,  $H^3$ -noradrenaline was selectively distributed in areas which contained high concentrations of catecholamines, the highest levels occurred in the hypothalamus and the lowest in the cerebral cortex and cerebellum (60). However, considerable amounts of  $H^3$ -noradrenaline were present in the corpus striatum, which normally contains high levels of dopamine and little endogenous noradrenaline. In autographic studies intense labeling was also found in the periventricular and ventromedial nuclei of the hypothalamus, medial forebrain bundle, in specific tracts of the spinal cord and in the apical dendritic layer of the hippocampus. Subcellular distribution studies, using continuous sucrose gradients, showed the  $H^3$ -noradrenaline, after its intraventricular administration in the brain, was present in the synaptosomal layer (pinched off nerve endings) together with endogenous noradrenaline (61). These observations indicated that  $H^3$ -noradrenaline mixed to a considerable degree with the endogenous brain stores of the catecholamine. The  $H^3$ -noradrenaline persisted in the brain for long periods of time, indicating that it was stored and protected from metabolism. The radioactive metabolites formed were normetanephrine and O-methylated deaminated metabolites. The major product in the brain was  $H^3$ -3-methoxy-4-hydroxyphenylglycol.

Labeling of the brain stores of noradrenaline provided an opportunity to study the effects of drugs on the uptake, storage, release and metabolism of noradrenaline in the brain (62). It was previously shown that imipramine and chlorpromazine blocked the uptake of  $H^3$ -noradrenaline in intact peripheral tissues (51) and brain slices (42). In the intact rat brain, imipramine reduced the accumulation of  $H^3$ -noradrenaline after its intraventricular injection (63), while chlorpromazine did not (Table 3). Other antidepressant

Table 3. Antidepressant Drugs and the Inhibition of Uptake of H<sup>3</sup>-Noradrenaline in the Rat Brain

Treatment	Clinical Antidepressant Action	H <sup>3</sup> -Noradrenaline gm/brain cpm × 1000
None		30 ± 2.0
Imipramine	Yes	19 ± 1.0 <sup>1</sup>
Desmethylimipramine	Yes	19 ± 1.1 <sup>1</sup>
Amitriptyline	Yes	23 ± 2.1 <sup>2</sup>
Compound 2	No	30 ± 1.6
Compound 3	No	28 ± 1.2
Chlorpromazine	No	32 ± 3.1

<sup>1</sup>P < .001

<sup>2</sup>P < .05

Groups of 6 rats were given drugs (20 mg/kg) intraperitoneally 1 hour before the administration of 0.07 μg of H<sup>3</sup>-noradrenaline into the lateral ventricle of the brain. Rats were killed two hours later and assayed for H<sup>3</sup>-noradrenaline. Compound 2 had the same structure as imipramine except that a dimethyl isopropyl side chain was substituted for a dimethylaminopropyl side chain. Compound 3 had the same structure as chlorpromazine except that a dimethylaminoethyl ether side chain was substituted for a dimethylaminopropyl side chain (63).

drugs such as desmethylimipramine and amitriptyline reduced the accumulation of H<sup>3</sup>-noradrenaline in the brain, but structurally related derivatives of imipramine which are clinically inactive as antidepressants had no effect. Both monoamine oxidase inhibitors and imipramine are antidepressant drugs and cause an increased amount of physiologically active noradrenaline to react with the adrenergic receptors in the brain. Each of these compounds makes more noradrenaline available in the brain by different mechanisms. Imipramine and other tricyclic antidepressant drugs slow inactivation by reuptake into the neurone, and monoamine oxidase inhibitors prevent metabolism of the catecholamine. Amphetamine has multiple actions on the disposition of the catecholamine in the brain (62). Like tricyclic antidepressants, it blocks uptake into the neurone, causes release of the catecholamine from its storage site, and inhibits monoamine oxidase. Amphetamine administration results in an increased formation of H<sup>3</sup>-normetanephrine in brain, whereas reserpine causes an increase in deaminated metabolites. These metabolic changes reflect a release from the neurone of physiologically active noradrenaline by amphetamine and a release of inactive metabolites by reserpine.

Glowinski and Iversen performed a study on metabolism of noradrenaline in different brain regions. They found that all areas of the brain except the striatum can convert dopamine to noradrenaline (64). Amphetamine blocked the reuptake of noradrenaline in all brain areas, whereas desmethylimipramine inhibited uptake in cerebellum, medulla oblongata and hypothalamus, but not in the corpus striatum (62). Rates of turnover of brain noradrenaline were

also examined by such different experimental approaches as measuring rates of disappearance of endogenous noradrenaline after inhibiting catecholamine biosynthesis, estimating rates of disappearance of  $H^3$ -noradrenaline formed from  $H^3$ -dopamine, and determining rates of disappearance of  $H^3$ -noradrenaline after its intraventricular injection. These methods produced results in close agreement with one another. Cerebellum had the fastest turnover and the medulla oblongata and hypothalamus had the slowest turnover (65). With these techniques subsequent work has established that turnover of brain noradrenaline is altered by a variety of stresses, temperature changes and sleep.

#### REGULATION OF THE BIOSYNTHESIS OF CATECHOLAMINES

The catecholamines are synthesized as shown in Fig. 7. This biosynthetic pathway was first proposed by Blaschko in 1939 (66) and finally established by Udenfriend and his coworkers (67). The first step is catalyzed by the enzyme tyrosine hydroxylase (67), the second by dopa decarboxylase (68), and the third by dopamine- $\beta$ -oxidase (69). These reactions occur within the sympathetic nerve terminal. The final step is catalyzed by phenylethanolamine-N-methyltransferase and occurs almost exclusively in the adrenal medulla (30). In the adrenal gland the biosynthetic enzymes tyrosine hydroxylase, dopamine- $\beta$ -oxidase and phenylethanolamine-N-methyltransferase are confined almost entirely to the adrenal medulla.

Noradrenaline in sympathetic nerves and catecholamines (noradrenaline and adrenaline) in the adrenal medulla are in constant flux. They are continuously being released, metabolized, and synthesized, yet they maintain a remarkably constant level in tissues. Recent work in our laboratory and those of others revealed several mechanisms that regulate the biosynthesis of catecholamines, involving long-term hormonal controls as well as short and long-term neural regulation.

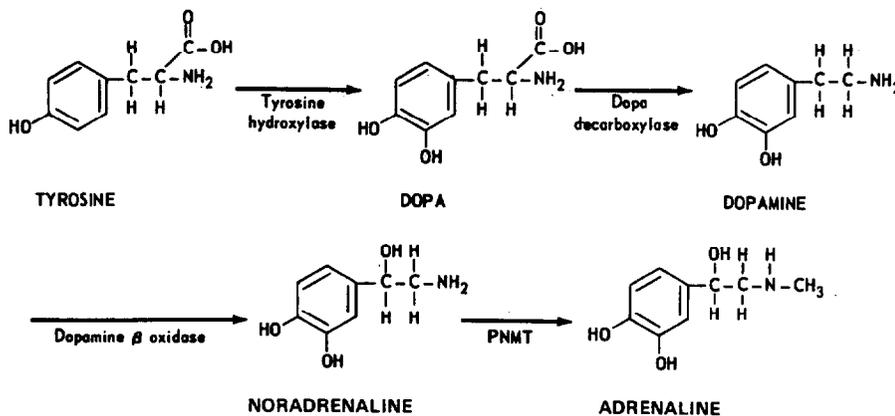


Fig. 7. The biosynthesis of catecholamines. PNMT is phenylethanolamine-N-methyltransferase.

## HORMONAL CONTROL

In species such as dogfish, where the chromaffin tissue is located outside the adrenal gland, little or no adrenaline occurs (70). In species where the medulla is completely contiguous with the cortex (human and rat) almost all of the catecholamine content is adrenaline. This suggested to Wurtman and me (71) that the adrenal cortex might affect the activity of the adrenaline forming enzyme phenylethanolamine-N-methyltransferase. I had been working on the properties of this enzyme and developed a sensitive and specific assay for its measurement (30). In the initial experiment we measured the effect of hypophysectomy on the phenylethanolamine-N-methyltransferase in the adrenal gland (71). The hypophysectomized rats showed steady fall of the adrenaline-forming enzyme until about 20 percent of the initial concentration remained (Fig. 8). The daily administration of either ACTH (Fig. 8) or dexamethasone for 21 days restored enzyme activity to normal levels in hypophysectomized rats. To examine whether the corticoid-induced rise in PNMT was due to increased synthesis of new enzyme protein, dexamethasone was given to rats whose RNA dependent protein synthesis had been inhibited by puromycin or actinomycin D. Both inhibitors of protein synthesis prevented the rise of enzyme activity caused by dexamethasone. However, repeated administration of ACTH or dexamethasone to intact rats failed to elevate adrenal PNMT activity above normal levels.

In view of the effect of hypophysectomy on the adrenal PNMT activity, the effect on other catecholamine biosynthetic enzymes was examined. After hypophysectomy there was a fall of adrenal gland tyrosine hydroxylase (72).

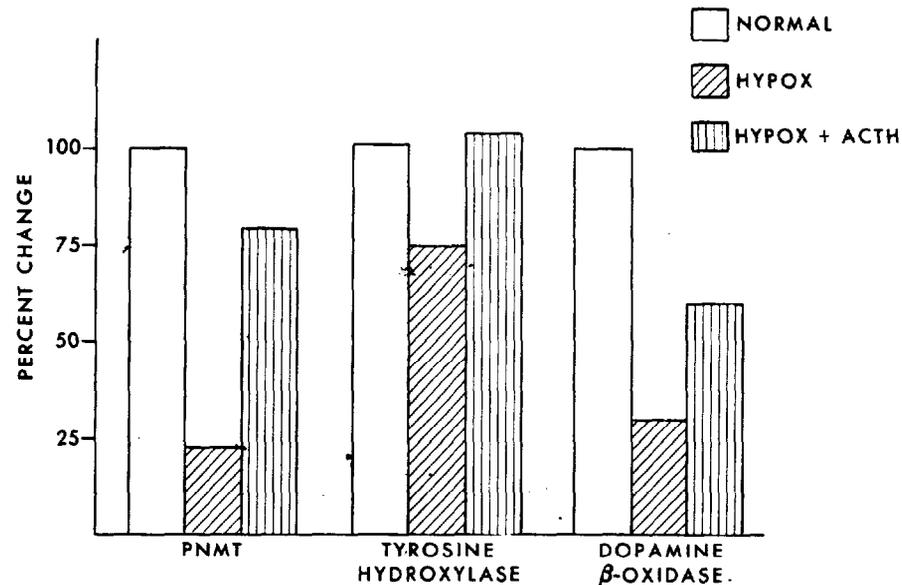


Fig. 8.

Control of enzymatic synthesis of adrenaline in the adrenal medulla by ACTH. Phenylethanolamine-N-methyltransferase (PNMT) (71) and dopamine- $\beta$ -oxidase (73) were measured 21 days after hypophysectomy, and tyrosine hydroxylase 5 days after hypophysectomy (72). ACTH was given, after hypophysectomy, daily for 6 days.

(Fig. 8). Enzyme activity was reduced 25 percent in 5 days (Fig. 8) and to about half in 10 days. Repeated administration of ACTH restarted tyrosine hydroxylase activity to normal values in hypophysectomized rats (Fig. 8). In contrast to PNMT, dexamethasone did not elevate tyrosine hydroxylase activity in hypophysectomized rats. Again repeated doses of large amounts of ACTH did not increase adrenal tyrosine hydroxylase in normal rats.

Dopamine- $\beta$ -oxidase (the enzyme that converts dopamine to noradrenaline) activity was also examined in hypophysectomized rats (73). This enzyme decreases to about 30 percent of normal values after 21 days (Fig. 8). Administration of ACTH for 5 days caused dopamine- $\beta$ -oxidase activity to increase, but full activity was not reached in this period of time. These observations indicate that the normal maintenance of the catecholamine biosynthetic enzymes in the adrenal glands requires ACTH.

#### NEURAL REGULATION

The biosynthesis of catecholamines in the sympathetic nerves and the adrenal gland is under precise control by nervous mechanisms, one of which is rapid and the other slower. After prolonged stimulation of the splanchnic nerve the sum of the amount of catecholamines released together with the amount remaining in the gland is greater than that initially present in the gland (74). This indicated that nerve impulses increases the biosynthesis of catecholamines. Weiner and his coworkers using an isolated preparation of the hypogastric nerve of the vas deferens showed that stimulation resulted in an increased synthesis of  $C^{14}$ -noradrenaline from  $C^{14}$ -tyrosine, but not from  $C^{14}$ -dopa (75). They also found that addition of noradrenaline prevented an increase in  $C^{14}$  catecholamine formation from  $C^{14}$ -tyrosine. However, stimulation of the vas deferens did not change the total amount of noradrenaline or tyrosine hydroxylase in vitro. The fact that noradrenaline is capable of inhibiting the conversion of  $C^{14}$ -tyrosine to noradrenaline indicated a rapid feedback inhibition at the tyrosine hydroxylase step.

Another type of regulation of catecholamine biosynthesis was uncovered in an unexpected manner. Tranzer and Thoenen reported that 6-hydroxydopamine selectively destroyed sympathetic nerve terminals (76). Thoenen decided to spend a sabbatical year in my laboratory, and together with Mueller we examined the effect of chemical destruction of sympathetic nerve terminals by 6-hydroxydopamine on the biosynthetic enzyme tyrosine hydroxylase. As expected, the enzymes completely disappeared within two days after the administration of 6-hydroxydopamine (77). However, when the adrenal gland was examined a marked increase in tyrosine hydroxylase was observed. Since 6-hydroxydopamine lowers blood pressure, the increase in enzyme activity caused by this compound might be due to a reflex increase in sympathetic adrenal activity. Consequently we examined the effect of reserpine, which is known to reduce blood pressure and increase preganglionic neuronal activity. Reserpine produced a marked increase in tyrosine hydroxylase activity over several days in the adrenal gland of the rat and several other species, in

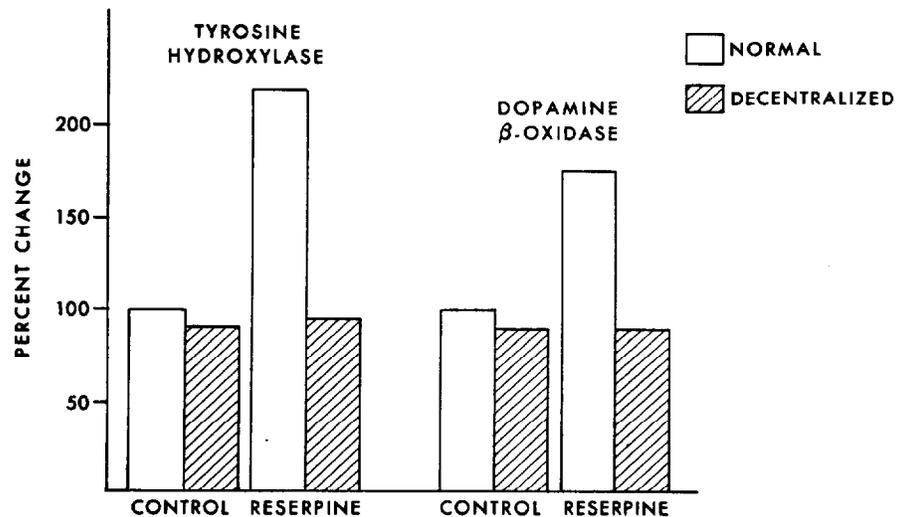


Fig. 9.

Transsynaptic induction of noradrenaline biosynthetic enzymes. Right or left superior cervical ganglion was decentralized by transection of the preganglionic trunk from 2 to 6 days before reserpine treatment. Reserpine (5 mg/kg) was given 24 hours before tyrosine hydroxylase was assayed in innervated and decentralized ganglia (78). In the case of dopamine- $\beta$ -oxidase, reserpine (2.5 mg/kg) was given on 3 alternated days and enzyme examined on the 7th day after decentralization (83).

the superior cervical ganglion (Fig. 9) and in the brainstem of the rabbit (78, 79). The adrenergic blocking agent phenoxybenzamine also caused a reflex increase in sympathetic adrenal activity. And again the administration of this compound resulted in an elevation in tyrosine hydroxylase activity in the adrenal gland. To examine whether the increased enzyme activity is due to the formation of new enzyme molecules, protein synthesis was inhibited prior to the administration of the drugs. Inhibition of protein synthesis with either cycloheximide or actinomycin D prevented the drug-induced increase of tyrosine hydroxylase in the adrenal gland and ganglia (80). The most likely mechanisms for the increase in enzyme activity might be a blood-borne factor, as in the induction of PNMT by ACTH, or an increase in the activity of the preganglionic neurones. To examine the latter possibility, we cut unilaterally the splanchnic nerve supplying the adrenal gland (81) and preganglionic fibers to the superior cervical ganglion and then administered reserpine (78). This drug caused the expected rise in tyrosine hydroxylase in the innervated side but the increase in tyrosine hydroxylase on the denervated side was completely prevented (Fig. 9). These results indicate that the increase in tyrosine hydroxylase is due to a transsynaptic induction of the enzyme. Studies on the molecular mechanisms that cause this induction across nerves have thus far proved unsuccessful. The neuronally-mediated induction of tyrosine hydroxylase after reserpine is also observed in the nerve terminals as well as the cell body. However, the increase in tyrosine hydroxylase in the nerve terminals lags behind the ganglia by two or three days (82). Experiments with inhibitors of protein synthesis point to a local formation of induced

tyrosine hydroxylase in the nerve terminals rather than the peripheral movement of the completed enzyme.

Similar studies on the induction of dopamine- $\beta$ -oxidase, an enzyme present in the noradrenaline storage granule, were undertaken with the collaboration of Molinoff, Weinshilboum and Brimijoin (83). These experiments were made possible because a very sensitive assay for dopamine- $\beta$ -oxidase was developed. This enzyme could be measured where it never has been found before. Repeated administration of reserpine caused a marked rise in dopamine- $\beta$ -oxidase in rat stellate and superior cervical ganglia (Fig. 9) in the nerve terminals as well as in the adrenal medulla. The elevation of dopamine- $\beta$ -oxidase in sympathetic ganglia was blocked by protein synthesis inhibitors or by surgical decentralization (Fig. 9). Recently we have found that dopamine- $\beta$ -oxidase is present in the plasma of man and other mammalian species (84). Preliminary experiments indicate that the circulating dopamine- $\beta$ -oxidase comes from sympathetic nerve terminals. The activity of PNMT in the adrenal gland also increased after reserpine and this elevation in enzyme was blocked by interrupting the splanchnic nerve (85).

Because of the increasing implications of catecholamines in behavioral changes we examined the effects of psychosocial deprivation and stimulation on the biosynthetic enzymes tyrosine hydroxylase, and PNMT (86). One group of mice was isolated to prevent visual contact and another was exposed to increased social stimulation by a specially designed cage system. After six months a marked decrease in adrenal tyrosine hydroxylase and PNMT activity occurred in the deprived mice and an increase in both these enzymes was found in the stimulated mice. In related experiments in Kopin's laboratory it was also observed that prolonged forced immobilization in rats also produced a rise in adrenal tyrosine hydroxylase and PNMT activities, and this elevation was abolished by interrupting the splanchnic nerve to the adrenal (87). All these results suggest that the increase in the catecholamine-forming enzymes in sustained stress may be neuronally mediated and that this response is not immediate, as in the case of a sudden discharge of noradrenaline and adrenaline in states of anger, fear or aggression.

#### NORADRENALINE AS A NEUROCHEMICAL TRANSDUCER IN THE PINEAL GLAND

The pineal gland is exceedingly rich in sympathetic nerve fibers which originate in the superior cervical ganglia (88). This organ has the unique capacity to synthesize the hormone melatonin (5-methoxy-N-acetyltryptamine) as follows: tryptophan  $\rightarrow$  5-hydroxytryptophan  $\rightarrow$  serotonin  $\rightarrow$  N-acetylserotonin  $\rightarrow$  melatonin (89). A year before the discovery of melatonin by Lerner (90) we found an O-methylating enzyme, COMT (7). This stimulated a search for the enzyme that O-methylates indoles to form melatonin. Such an enzyme was found in the pineal gland and named hydroxyindole-O-methyltransferase (29). The enzyme O-methylates N-acetylserotonin to form melatonin, S-adenosylmethionine serving as the methyl donor. At about the same time the enzyme (N-acetyltransferase) that acetylates serotonin to N-acetylserotonin was described

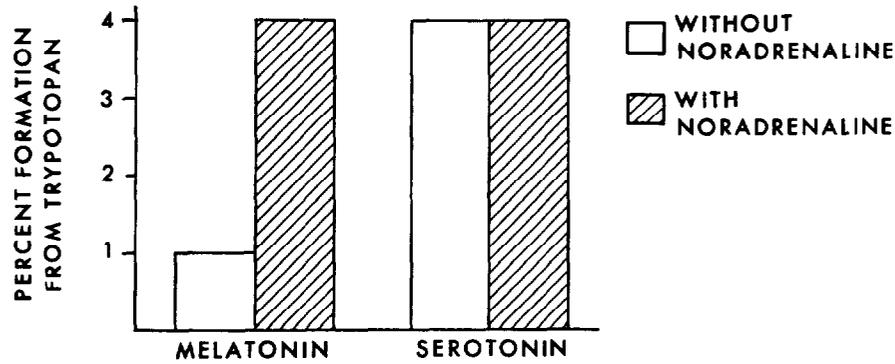


Fig. 10.

Stimulation of melatonin synthesis in pineal gland by noradrenaline. Culture tubes of pineal gland of rats were incubated with  $C^{14}$ -tryptophan in the absence or presence of noradrenaline ( $3 \times 10^{-4}$  M). After 24 hours the pineal cultures were assayed for  $C^{14}$ -serotonin and  $C^{14}$ -melatonin (97).

(91). The latter enzyme subsequently proved to be critical in the control of melatonin by the adrenergic nervous system. That environmental lighting had something to do with the pineal was suggested by Fiske in 1961 (92) when she found that continuous light changed the weight of the organ. Consequently rats were placed in continuous darkness or light and activity of the melatonin forming enzyme hydroxy indole-O-methyltransferase in the pineal was measured (93). In constant darkness the hydroxyindole-O-methyltransferase activity in the pineal was more than twice as great as that in constant light. Removal of the superior cervical ganglia abolished this difference in enzyme activity (94). Since noradrenaline is the neurotransmitter of the sympathetic nerves, it might be the agent involved in controlling melatonin synthesis. This possibility was reinforced by the demonstration that levels of noradrenaline in the pineal are markedly influenced by environmental lighting (95). A possible approach to an examination of the mechanism whereby the neurotransmitter could influence the synthesis of melatonin (which occurs outside the neurone) was to use pineals in organ culture. Mainly through the efforts of Shein, we succeeded in growing pineal gland in organ culture. The pineal in organ culture was capable of carrying out all the steps in the formation of melatonin from tryptophan (96). Inhibition of protein synthesis completely stopped the conversion of tryptophan to melatonin, indicating that new enzyme protein was being formed. Addition of noradrenaline to the culture medium resulted in a sharp increase of melatonin, but not serotonin formation from tryptophan over a period of 24 hours (97). (Fig. 10). However, noradrenaline had only a marginal effect on the hydroxyindole-O-methyltransferase activity. Klein et al. examined the enzyme that converts serotonin into N-acetylserotonin in pineal organ culture. He found that noradrenaline causes remarkable increase in the activity of this enzyme (98). When protein synthesis was blocked, noradrenaline no longer stimulated the N-acetyltransferase activity. These results show that noradrenaline released from sympathetic

nerves stimulates the formation of the pineal hormone melatonin by specifically increasing the synthesis of new N-acetyltransferase molecules.

#### CONCLUDING REMARKS

Since the demonstration by Otto Loewi (99) that sympathetic nerves exert their effects by the release of a chemical substance, numerous advances have occurred. The neurotransmitter has been identified as noradrenaline and its biosynthesis, metabolism and inactivation elucidated. Although the complexities of the storage, release and regulation of noradrenaline and adrenaline have been partially unravelled, much remains to be done. Our understanding of central adrenergic mechanisms is still at the early stages but shows great promise for rapid development. Drugs therapeutically effective in the treatment of affective disorders and neurological and cardiovascular diseases have also been shown to influence the uptake, storage, release, formation and metabolism of catecholamines. These findings implicating the peripheral and central sympathetic nervous system have provided insight into the causes and treatment of mental depression (100), Parkinson's disease (101) and hypertension (102).

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